A METHOD OF ENGINEERING DRUG-SPECIFIC HYPERSENSITIVE T-CELLS FOR IMMUNOTHERAPY BY GENE INACTIVATION

FIELD OF THE INVENTION

[0001] The present invention relates to the use of therapeutic cells for cell therapy or immunotherapy to treat patients in need, such as affected by cancer. In particular, the invention provides with a method of engineering immune cells (e.g. CAR T.-cells) by gene inactivation to render them sensitive to number of approved drugs. In case of adverse events or excessive immune response, these drugs can be administrated to the patient to deplete such engineered cells playing the role of "switch off" or "moderator". The invention opens the way to safer adoptive immunotherapy strategies for treating cancer.

BACKGROUND OF THE INVENTION

[0002] Adoptive immunotherapy, which involves the transfer of autologous or allogeneic antigen-specific immune cells generated ex vivo, is a promising strategy to treat cancer. The immune cells used for adoptive immunotherapy can be generated either by expansion of antigen-specific cells or redirection of such cells through genetic engineering (Park, Rosenberg et al. 2011). Transfer of viral antigen specific cells is a well-established procedure used for the treatment of transplant associated viral infections and rare viral-related malignancies. Similarly, isolation and transfer of tumor specific immune cells, in particular T-cells, has been shown to be successful in treating melanoma. Novel specificities in immune cells have been successfully generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (CARs). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains in a single fusion molecule. CARs have successfully allowed T-cells and NK cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors (Jena, Dotti et al. 2010).

[0003] T cell adoptive immunotherapy which involves the transfer of antigen-specific T-cells generated ex vivo, is a promising strategy to treat cancer. The T-cells used for adoptive immunotherapy can be generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (CARs). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains. CARs have successfully allowed T-cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors. However, despite their unprecedent efficacy for tumor eradication in vivo, CAR T cells can promote acute adverse events after being transferred into patients. On another hand, it would be desirable for doctors to have the possibility to reduce the engineered cells count in-vivo to modulate the immune response in accordance with the biological tests and monitoring performed on the patient during the course of the treatment. Among the potential adverse events are Graft versus host disease (GvHD), on-target off-tumor activity or aberrant lymphoproliferative capacity that may be due, among others, to vector derived insertional mutagenesis.

[0004] Thus, there is a need to develop cell specific depletion systems to prevent deleterious events to occur or to reduce the engineered cell count in vivo after engraftment of cells into a patient. Here, the inventors have thought about endowing engrafted cells with hypersensitivity properties toward a specific drug as an efficient solution to confer drug hypersensitivity to allogeneic cells.

[0005] The present invention relates on gene editing approaches, particularly adapted to immune primary cells, to create, or increase a pre-existing, sensitivity of the cells to approved drugs, to allow the depletion of said cells in response to said drugs during the course of a cell therapy.

SUMMARY OF THE INVENTION

[0006] In a general aspect, the present invention provides with methods of producing ex-vivo human cells, preferably immune cells, such as T cells, that can be depleted in-vivo as part of a cell therapy or immunotherapy treatment. Such treatment typically comprises a step of inducing a drug hypersensitivity into said human, preferably into immune cells, by selectively inactivating or inhibiting the expression of one gene involved in the metabolization, elimination or detoxification of said drug.

[0007] The inventors have sought for the inactivation of a selection of such gene, which actually conferred drug-specific hypersensitivity to engineered cells. In particular, inactivation of RhoA gene could be performed to genetically engineer human cells, preferably immune cells, in order to make them hypersensitive to doxorubicin. The inactivation of CDK5 gene conferred cell hypersensitivity to bortezomib. The inactivation of CXCR3, NR1H2, URG4, PARP14, AMPD3, CCDC38, NFU1 and/or CACNG gene conferred cell hypersensitivity to neratinib. The inactivation of GGH conferred hypersensitivity to 5-FU and resistance to methotrexate. The inactivation of SAMHD1 conferred hypersensitivity to deoxycytidine analogs, such as cytarabine (ara-C). These inactivations were effective in primary immune cells, in particular CAR T-cells used in immunotherapy.

[0008] The inhibition of expression of such gene(s) is preferably performed by gene editing, and in particular by introducing into said human cells at least one engineered rare-cutting endonucleases targeting said gene, such as TALE-nuclease, Zing Finger nuclease, RNA-guided endonuleases (e.g. Cas9 or Cpf1), Argonaute, or homing endonuclease.

[0009] The resulting drug-hypersensitive cells, especially the immune cells, can be further engineered to express a Chimeric Antigen Receptor (CAR) to direct their cytotoxicity towards unwanted cells (ie. tumoral cells) expressing particular surface antigen markers. Such engineered cells can be also modified to be less alloreactive by inactivating genes involved into the expression of T cell receptor (e.g. TCRalpha or TCRbeta) in view of their safer use in allogeneic treatment. Further genes may also be transiently or definitely inactivated such as those expressing immune-checkpoints (e.g. PD1)) to improve the tolerability of the engineered cells by the host organism.

[0010] The present invention relates also to an isolated human cell, preferably immune cell, made hypersensitive to a drug obtainable by the above method, a pharmaceutical composition containing same for its use in the treatment of cancer, infection or immune disease.

[0011] Furthermore, the present invention concerns the use of at least one isolated human cell, preferably immune